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Triggering of a Cytolytic Factor with TNF-Like Activity from Human Monocytes

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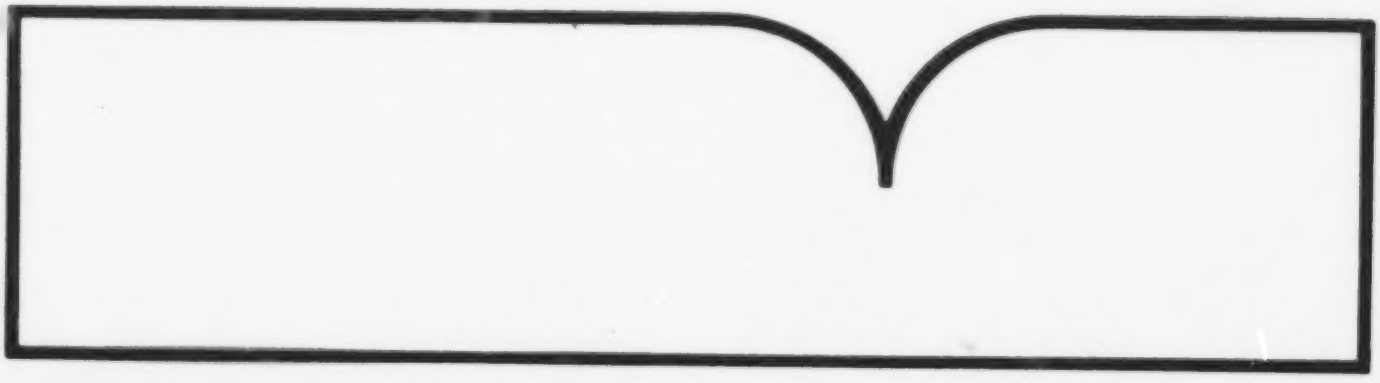

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TRIGGERING OF A CYTOLYTIC FACTOR WITH TNF-LIKE ACTIVITY FROM HUMAN MONOCYTES

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16. ABSTRACT

Monocytes have a nonoxidative mechanism of tumor cytolysis operative in CGD and in normal monocytes under anaerobic conditions. The absence of competitive inhibition provides negative evidence for a contact-independent mechanism of cytolysis, confirmed by the demonstration of a soluble mediator secreted independently of effector-target cell contact. This cytolytic monokine, a protein of apparent molecular weight between 25,000 and 40,000, resembles TNF functionally and antigenically. Further work will be required to demonstrate its biochemical identity, its precise mechanism of toxicity, and the extent of its importance in vivo.

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1. INTRODUCTION

Mononuclear phagocytes (MNP) have been implicated in host defense mechanisms against tumors (1), in addition to well-established roles as scavengers (2), microbicidal effectors (3), antigen-processing and presenting cells (4), and secretors of immunoregulatory mediators (5,6). Evidence that MNP participate in tumor surveillance has recently been extensively reviewed (7). Macrophage (Mø) function is deficient in old age, immunosuppression, and immunodeficiencies, conditions associated with both decreased immune function and increased cancer incidence. Stimulation of Mø function can decrease tumor incidence (8). MNP may also reject established tumors. Murine (9,10,11) and human (12,13) MNP have been observed to infiltrate tumors. Mø's capable of lysing tumor targets have been isolated from tumors and ascitic fluid (14-16). Such Mø's may affect the course of disease because, in the mouse, higher Mø tumoricidal activity is associated with regression and dormancy of certain tumors, and in humans, greater tumor infiltration is associated with fewer metastases (17). Furthermore, some tumors regress when injected with agents that activate Mø's (18-20).

Cytotoxicity against tumor cells mediated by Mø's *in vitro* was demonstrated first by using Mø's obtained from rodents either immunized with the homologous tumor cell line (21), injected with protozoa (22) or treated with lipopolysaccharide (LPS) (23). Hibbs (24,25,26) demonstrated that embryo fibroblast cytolysis was independent of H-2 compatibility and correlated instead with spontaneous transformation *in vitro*, and furthermore, that loss of contact inhibition and acquisition of tumorigenicity predicted susceptibility to lysis better than aneuploidy, continuous growth, or

abnormal morphology. This selectivity for neoplastic cells has been confirmed repeatedly in rodent Mø systems (e.g., 27,28). Similar selectivity has been observed for cytolysis and cytostasis by human monocytes (e.g., 29,30,31,32) and Mø's (33), even when normal and transformed targets are mixed (34). Although the selectivity of mononuclear phagocytes (MNP) for transformed targets is now well accepted, some studies indicate that this pattern is not absolute (35,36,37).

Alexander and Evans (23) showed that 2.5 to 25 µg/ml doses of lipopolysaccharide (LPS) or lipid A *in vitro* or *in vivo* render murine peritoneal Mø's cytotoxic for L5178Y lymphoma targets. By using ^{51}Cr-release assays, Doe and Henson (38) showed that LPS-activated Mø's are not only cytostatic but actually lyse tumor cells. Furthermore, these authors have shown that LPS acts on purified Møs via the lipid A portion of the molecule (40). The first evidence for a two-stage activation sequence for murine Mø's for tumoricidal activity was that Mø's isolated from *M. bovis* strain BCG- or toxoplasma-infected mice are cytolytic when tested in fetal calf serum (FCS), but not in human sera unless 1 to 5 ng/ml LPS is added, a dose of LPS which does not activate normal Møs for cytotoxicity (41). It was soon confirmed that murine Mø's activated *in vivo* by BCG infection or *in vitro* by macrophage-activating factor (MAF) also require triggering for cytotoxicity, but the quantity of LPS required is only on the order of 1 ng/ml (41), an amount often present as an inadvertant contaminant of media and sera (41). The synergistic effect of MAF and LPS requires MAF treatment before or contemporaneous with LPS treatment (42).

There is not a similarly well understood activation pathway for human MNP. There have been several reports of spontaneous cytotoxicity

(defined as cytotoxicity without intentional activation) against tumor cells mediated by human peripheral blood monocytes (e.g., 43), cultured monocyte-derived macrophages (44,45), and Mø's (46,47,48). Fresh adherence-purified monocytes have been demonstrated spontaneous tumoricidal activity (49,50,51) even when LPS contamination is excluded (51,52). Several studies indicate that freshly isolated human monocytes are activated by various lymphokines (LK) or interferon (IFN) preparations (53-57). However, the potential contribution of natural killer (NK) cell contamination (58,59) poses particularly severe problems of interpretation of these studies (60) and the possibility of LPS contamination was seldom excluded.

As far as the killing mechanism of tumor cells by MNP is concerned, several lines of evidence in both murine (21,23,26,31) and in human systems (54), indicate that binding plays an important role in target selectivity by MNP. Activation of murine Mø's and human monocyte-derived Mø's is associated with increased binding activity. Moreover, prevention of binding of activated murine Mø's by means of trypsinization or addition of a chelating agent or unlabeled target cells to the assay abolishes cytotoxicity (61). Contact dependence of cytotoxicity does not exclude the possibility that the mediators of cytotoxicity may be soluble. Cell contact may be required to trigger directed secretion of a cytotoxic mediator. A chemically unstable cytotoxic mediator may act only in the immediate vicinity of effector cells. Electron microscopic observations suggest that the zone of contact between an effector and a target cell may be narrow enough to limit diffusion of macromolecules in and out of the zone (62). Therefore, the attachment of an effector to a target cell may allow the accumulation of a

sufficiently high local concentration of a soluble mediator, or may prevent inactivation of the soluble mediator by inhibitors.

MNP secrete a large variety of molecules (63,64). MNP products reported to have selective cytotoxic effects on neoplastic cells include lysozyme (65), IFN (66), C_3A (67), Thymidine (68), and hydrogen peroxide (69,70). The role of thymidine and C_3A at physiologic rates of production has been disputed. Hydrogen peroxide and other reactive oxygen species, whose role in microbial defense is well established (71), have received the most attention.

Several observations suggest that the oxidative burst may mediate monocyte tumoricidal activity. Mammalian cells are sensitive to lysis by reactive oxygen species (72). Moreover, lysis by peroxide is rapid enough (73) to account for the kinetics of antibody-dependent cellular cytotoxicity (ADCC) and antibody-independent cytotoxicity by fresh peripheral blood monocytes (43,74). MNP efficiently generate reactive oxygen metabolites (75,76). Moreover, Mø's activated for cytotoxicity by IFN-gamma, bacterial products such as LPS and muramyl dipeptide, and inflammatory proteases have increased levels of oxidative burst activity (77-80). Also, fresh blood monocytes have relatively high oxidative burst potential which declines gradually in culture (81), roughly in parallel with their loss of spontaneous cytotoxic potential (55).

Nathan and co-workers (69,70) have shown that lysis of neoplastic targets by activated murine macrophages stimulated by phorbol myristate acetate (PMA) depends on hydrogen peroxide production. These observations have been extended to fresh human peripheral blood monocytes stimulated by PMA (82-85). Although these studies demonstrate that the oxidative burst

of monocytes and macrophages can lyse neoplastic cells, they do not establish that the oxidative burst does mediate tumor lysis under physiologic conditions. Indeed, Weinberg and Haney (84) found that scavengers of reactive oxygen species could not inhibit spontaneous cytolysis of HeLa target cells by unstimulated human monocytes or peritoneal Mø's. These results suggest that reactive oxygen species play no role in cytolysis unless the oxidative burst is stimulated extrinsically. However, it is conceivable that in these experiments, the scavengers of reactive oxygen intermediates inhibited monocyte/macrophage mediated lysis, and NK contaminants accounted for at least part of the observed lytic activity. Another possibility is that hydrogen peroxide mediates lysis both with and without PMA, but without extrinsic stimulation, the effects of hydrogen peroxide are confined to a contact zone from which catalase is excluded (85).

An alternative approach to the problem of defining lytic mediators now appears more promising. Several groups have detected cytotoxic activity in the supernatants of activated Mø's (86-94), and monocytes (95-97).

Currie and Basham (89) showed that activated murine macrophages release a factor that selectively lyses malignant, but not normal, cells. Subsequently, Currie showed that the factor has arginase activity that accounts for its cytotoxicity (98). Although this observation has been confirmed in the mouse (99), it has not been extended to humans. It is also questionable whether in vivo arginine could be depleted to cytolytic levels.

Adams and coworkers have demonstrated that one cytolytic mechanism of activated murine Mø's is secretion of a cytolytic protease (100). They showed that: (a) activated murine macrophages secrete a neutral protease;

(b) serum-free supernatants of activated Mø's lyse a spectrum of neoplastic targets; and (c) inhibitors of serine proteases inhibit lysis both by supernatants and by Mø's; therefore, secretion of protease is one cytolytic mechanism of activated Mø's (100). They have also shown that secretion of the cytolytic protease is a property of murine Mø's that have been fully activated in vivo (86) or in vitro (101). Cytolytic protease secretion is increased by binding tumor targets or target membrane preparations (102).

Reidarson has described actived murine Mø's cytotoxins (MCT) of molecular weight 150,000 and 60,000 daltons (92) whose activity, like cytolytic protease, is inhibited by serine protease inhibitors (93). MCT activity has been purified 2000- to 5000-fold (103). This group has isolated similar cytotoxins that can be inhibited by protease inhibitors from supernatants of a PMA-treated human monocytic leukemia cell line (104).

TNF is defined as the substance that causes necrosis and regression of certain tumors that is found in the sera of mice, rabbits, and rats primed with BCG and elicited with LPS (105). TNF activity as measured in vivo correlates with cytotoxicity to various tumor cell lines in vitro, suggesting that the same molecule mediates both in vivo and in vitro effects (105-107).

The mode of action of TNF is not known. The slope of the dose-response curve is shallower even against cloned targets than predicted for single-hit kinetics, suggesting that TNF may act enzymatically (108). O-phenanthroline, a metal chelator, blocks TNF killing by over 99%, suggesting that TNF may be a metalloenzyme. Sodium azide also chelates metals and inhibits action of TNF. Colchicine and chloroquine inhibit its effects, suggesting

that TNF must be endocytosed and lysosomal function is required for action (109). In addition to cytotoxicity against tumors, TNF may mediate resistance to malaria (110,111), and may regulate the activity of lipoprotein lipase in cultured adipocytes (112).

Matthews (113) demonstrated that human monocytes stimulated with LPS also produce a cytotoxin detected in TNF assays in vitro against L929. Weinberg (114) showed that HL-60, a human promyelocytic leukemia cell line, when treated with PMA, acquires cytolytic capacity. These cells also secrete a cytotoxin, whose production is stimulated by LPS, with physicochemical and functional properties similar to TNF (115). Recently, two groups have cloned cDNA for this cytotoxin, identified as TNF as defined by cytotoxicity in vitro and tumor necrosis in vivo (116,117).

McKinnon et al. (118) and Chen et al. (53) have recently shown that unstimulated human monocytes can produce a cytolytic factor against actinomycin D-treated WEHI-164 (WEHI/D) targets in line with the findings of Ziegler et al. (50). The cytolytic activity was shown by Colotta et al. (118) to be inhibited by serum protease inhibitors; furthermore, cytolysis by monocytes in cell-mediated assays is inhibited by macromolecular, but not micromolecular, protease inhibitors (119).

Hammerström et al. have described factors selectively cytostatic for transformed cells. These factors are produced by human monocytes cultured in vitro for four days, and then stimulated with LK and LPS (97). These factors are not cytolytic; they cause a reversible increase in cell-cycle time, inhibiting DNA synthesis earlier and more severely than protein synthesis (120,121).

In this chapter, we will discuss some of our recent findings as they relate to major issues in the area of MØ cytotoxicity mentioned above. The key questions that our studies addressed are as follows:

1) Is LPS an obligatory and sufficient trigger for the induction of human monocyte-mediated cytotoxicity?
2) What are some of the biological and biochemical characteristics of the monocyte-derived cytolytic factor?
3) What is the spectrum of target cells sensitive to the cytolytic factor, and what is the relationship between susceptibility to lysis and ability to trigger the production of cytolytic factor?

2. RESULTS AND DISCUSSION

2.1 The Role of LPS as a critical signal for triggering monocyte-mediated cytotoxicity.

It has recently been shown that WEHI-164 murine sarcoma tumor cells that have been pretreated with actinomycin-D (WEHI/D) are sensitive to human monocyte-mediated killing in a 6-hr ^{51}Cr-release assay, while they remain insensitive to lysis by natural killer (NK) cells (50,51). We have made use of this target cell to investigate the role LPS plays in cytolytic reactions (52). All the tissue culture reagents and cells used in those studies were prepared under LPS-free conditions (<0.05 ng/ml LPS as detected by the Limulus amebocyte lysate assay).

Monocytes prepared and assayed under sterile conditions had significant cytolytic activity against WEHI/D targets (Fig. 1). There was considerable variation in baseline cytolytic activity amongst donors. Invariably, however, addition of 10 ng/ml LPS directly to the tumoricidal assay resulted

in enhanced lysis ($n=11$, $p<0.001$, Wilcoxon two-tailed rank-sum test). The addition of polymyxin B at 10µg/ml to the assay inhibited the most active preparations of cytotoxic monocytes, though significant levels of cytolysis persisted in all 3 experiments. It should be mentioned that LPS by itself does not lyse WEHI/D cells, and monocytes pulsed with LPS and washed retain enhanced cytolytic activity. Therefore, the increase in cytolysis of WEHI/D in the presence of LPS was due to stimulation of the monocytes, not merely weakening of the target cells (52). Previous work has shown that LPS activates murine Mø's for cytostasis (122) and cytolysis (123) and that LPS stimulates cytotoxicity of human Mø's (48,124) and cultured monocyte-derived Mø's (43,125,126). However, previous studies of fresh monocytes revealed inconsistent and small (127) or no (84) effect of LPS. Our studies, therefore, represent the first unequivocal evidence that LPS augments tumor cytolysis by freshly isolated human monocytes. In the earlier studies, NK cells may have contributed significantly to the activity measured in these earlier studies (128) but do not lyse WEHI/D (50,51). Also, because the previous studies used long-term (48 to 60 hr) assays, the effector cells may have lost their capacity to respond to LPS as a result of maturation. In accordance with previous work (127,84), LPS was not absolutely required for expression of cytolytic activity, because 10 of 11 donors had significant levels of killing in the absence of LPS.

Titration of LPS added directly to the tumoricidal assay revealed a half-maximal effect at a dose of 100 pg/ml (Table 1). Because most endotoxin effects are mediated by the lipid A portion of the molecule, we tested a purified lipid A preparation in parallel with LPS. Lipid A alone was as effective as, but less potent than, intact LPS, with a half-maximal effect

at a dose between 100 pg/ml and 1 ng/ml (Table 1) suggesting that the polysaccharide chains of LPS may enhance binding. However, endotoxin preparations from different bacteria were equally effective despite having different polysaccharide structures. All LPS preparations tested, including polysaccharide-deficient LPS of the mutant S. typhimurium Re G30/31, had half-maximal effects at 100 pg/ml (Table 1) (52).

Because of the potential clinical value of a detoxified activating agent, we tested monophosphoryl lipid A in our assay. Although we confirmd that this preparation activated the Limulus amebocyte lysate assay, in 6/6 experiments, monophosphoryl lipid A caused no increase in cytolytic activity (52).

The half-maximal activity of LPS observed in our study is in accordance with the sensitivity of primed murine Mø's (129,130) but demonstrates much greater sensitivity than has been observed with the use of cultured human monocytes (125,126). Unlike murine Møs, however, fresh human monocytes did not require priming in vitro.

2.2 Monocyte killing of WEHI/D is contact-independent and the cytolytic factor is TNF-like.

Because excess unlabeled targets did not competitively inhibit cytolysis of WEHI/D by monocytes, studies were performed to examine the possible involvement of soluble factors (52,118). Supernatants from 5-hr cultures of fresh monocytes lysed WEHI/D as effectively as monocytes themselves. The kinetics of lysis by the cytolytic factor, which was termed cytolytic monokine (CM), were identical to those observed in direct cell-mediated assays (50,51). LPS added to monocyte cultures increased CM titers 100- to 1000-fold, so CM titers were sufficient to account for total cell-mediated

cytolysis both without LPS and at every dose of LPS tested. Taken together, these data demonstrate that monocytes lyse WEHI/D in a contact-independent manner by secretion of CM, and that the mechanism whereby LPS activates monocytes for cytolysis is to trigger secretion of CM. CM was found to be filtrable, heat labile, and trypsin sensitive (Table 2). The apparent m.w. of CM by S-200 column chromatography was between 25,000 and 40,000. Experiments using actinomycin D and cycloheximide revealed that release of CM requires both transcription and translation (Table 2).

Other investigators have described cytotoxic factors produced by human MNP stimulated by LPS (77,94,95). Because monocytes of a CGD patient produce high levels of CM (131), it is unlikely that oxygen intermediates are involved in lysis of WEHI/D. CM is unlikely to be a neutral serine protease because its activity even at high dilutions is unaffected by 10% FCS and by a mixture of protease inhibitors known to inhibit murine cytolytic protease (132). In contrast, antisera to recombinant human TNF neutralize CM activity (52, and Table 2). Together with the observation that recombinant TNF lyses WEHI/D, these data suggest that CM is antigenically and functionally similar, if not identical, to TNF. The human monocyte cytotoxin described by Matthews (95) is qualitatively similar to CM except that it is harvested at 20 hr, by which time CM activity has declined substantially. We also observed greater stimulation by, and sensitivity to, LPS than did Matthews, perhaps because we carefully excluded endotoxin contamination of our control monocyte preparations, or perhaps because of differences in our cytotoxic assays.

2.2 Target cell selectivity and capacity to induce CM production in monocytes

Regarding the target cell spectrum of CM, we have observed that several neoplastic targets, including K562, L929, and CEM, were lysed by some CM preparations (Table 3). No nonneoplastic target was lysed by CM, and not all neoplastic targets are susceptible to CM. Actinomycin D pretreatment of WEHI-164 and TU5 target cells enhanced the cytolytic effect. The observation of selectivity for a range of tumor cells supports the notion that CM may contribute to surveillance against tumor cells in vivo.

Murine Mø cytolytic factor (133) and human NK cytotoxic factor (NKCF) (134) can be stimulated by contact of effector and target cells. In experiments performed under LPS-free conditions, we examined the possibility that some target cells may induce monocytes to secrete CM.

To our surprise, enhanced CM activity was produced not by monocytes cultured with WEHI/D or K562, but by monocytes cultured with nontransformed lung cells (Fig. 2). There was no additive stimulation of CM production when monocytes were cultured with both LPS and any of the cell lines. The failure of monocyte-sensitive target cells to induce greater CM activity could have been explained by absorption of CM from the media by CM-susceptible cells. However, absorption experiments performed at 4°C (Fig. 3) or 37°C (not shown) did not reveal any reduction of CM activity from monocyte supernatants incubated 1 hr with WEHI-164, WEHI/D, TU5, TU5/D, L929, lung or autologous non-adherent lymphocytes. We therefore conclude that the lack of enhanced CM activity in supernatants produced by monocytes cocultured with WEHI/D was not due to a combination of stimulation and absorption by the susceptible targets.

Further experiments done with the normal lung cells showed that at ratios of 1 lung cell:100 monocytes, lung cells incubated with monocytes increased CM titers 16-fold. Similarly, TU5 and TU5/D cells at ratios of 1 Tu5 cell:10 monocytes increased CM titers 8-fold. This efficient stimulation suggested that the stimulation did not require cell contact. Indeed, monocytes incubated in conditioned media from 4-hr cultures of TU5 or lung cells produced supernatants with high levels of CM activity, although not as high as monocytes incubated directly with TU5 cells. Interestingly, conditioned media from TU5 and lung cells activated the Limulus amebocyte lysate, even though the cells had been cultured in endotoxin-free media; conditioned media from WEHI-164 cells, which did not stimulate CM production, did not activate Limulus lysate. Stimulator cells were not contaminated with mycoplasma, and still produced supernatants with stimulatory activity after being maintained in LPS-free media for months. Fig. 4 shows that whereas 10 µg/ml polymyxin B reduced LPS-stimulated CM titers from 31 to 6, polymyxin B did not significantly affect CM titers stimulated by conditioned media from lung or TU5 cells. There have been several demonstrations of false-positive Limulus tests for endotoxin caused by similar (135) and unrelated (136) molecules. The lack of inhibition of the stimulatory effect of lung and TU5 supernatants by polymyxin B, which binds the lipid A portion of the endotoxin molecule (137), suggests that these supernatants contained a stimulatory molecule other than LPS, the nature of which remains to be determined.

Although our studies do not rule out additional cytolytic mechanisms, they provide no evidence that cytolysis of WEHI/D by monocytes is enhanced by cell contact. Therefore, although many studies have demonstrated contact-dependent cytolysis by MNP (e.g., 28,29, 138), it is inappropriate

to extrapolate results obtained with MNP at a particular stage of maturation and activation, and one target to other stages of maturation, activation, and targets.

Conversely, the existence of a contact-independent lytic mechanism cannot be extrapolated to all targets. Recently, Keller (139) also described a contact-independent mechanism in a murine Mø cytotoxicity system. It should be noted that actinomycin D in our studies (data not shown) was not required for cytolysis by CM: monocyte supernatants also lysed TU5 and WEHI-164 in long-term ^{3}H-thymidine-release assays without drug treatment.

How CM works at the target cell level is unclear. The slope of the dose-response curve of CM is, as for TNF (108), shallower even against cloned targets than predicted for single-hit kinetics, suggesting that one CM molecule may kill more than one target, consistent with the hypothesis that it acts enzymatically. It is suggested that TNF may be a metalloenzyme because the metal chelator, o-phenanthroline, blocks TNF activity (109). The lack of absorption of CM activity by targets is consistent with external enzymatic action, but many other interpretations are possible. If CM binds a receptor, and is, like recombinant human TNF, effective at doses of 10^{-11} M, the lack of absorption by 2×10^{7} targets/ml of barely detectable activity indicates that fewer than 300 receptors/cell need to be occupied to effect lysis. Because actinomycin-D increases the susceptibility of target cells to CM, CM most likely causes damage that can be repaired by processes requiring transcription. A lack of hemolytic activity distinguishes CM from granules isolated from CTL (140) and NK (141) tumor cell lines, and suggests that membrane lipids are not the primary target of CM activity.

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TABLE 1

COMPARISON OF VARIOUS ENDOTOXIN PREPARATIONS TO INDUCE MONOCYTE-MEDIATED CYTOTOXICITY*

Endotoxin Preparation	Capacity to Induce Cytotoxicity	Half-Maximal Effect
LPS E. Coli 0111:B4	+	100 pg/ml
LPS S. Minnesota WT	+	100 pg/ml
LPS S. Typhimurium Re G30/C21	+	100 pg/ml
LPS S. Typhimurium WT	+	100 pg/ml
LPS S. Enteritidis	+	100 pg/ml
Lipid A	+	100 pg/ml-1 ng/ml
Monophosphoryl Lipid A	-	-

*Various endotoxin preparations were tested at 10 ng/ml in a 6 hr cytolytic assay of fresh monocytes against WEHI/D target cells.

TABLE 2

BIOCHEMICAL CHARACTERISTICS OF CM

CHARACTERISTIC	PROCEDURE	% OF CONTROL ACTIVITY
Heat stability	Boiling, 10 min	1
	56C, 30 min	1
Storage stability, 24 hr	37C	56.3
	4C	74.7
	-20C	45.0
Protease sensitivity	100 U trypsin, 90 min	4.4
Requirement for synthesis	10 ug/ml actinomycin D	1
	10 ug/ml cycloheximide	
Reactivity with rabbit anti-TNF antibodies	10 TNF-neutralizing units/ml	40.4

CHARACTERISTIC	PROCEDURE	RESULT
M.W.	Sephacryl S-200 Column	25-40Kd
Rate of Production	"Time window Experiment"	Highest between second and fifth hr.

TABLE 3

SUSCEPTIBILITY OF TARGETS TO CM AND THEIR ABILITY TO TRIGGER CM RELEASE

TARGET	SUSCEPTIBILITY TO LYSIS	CAPACITY TO STIMULATE CM PRODUCTION
Transformed Targets		
A375	-	-
A375/D	±	
CEM	-	
CEM/D	-	
K562	Variable	-
K562/D	-	
L929	Variable	
L929/D	Variable	
Natusch	-	
Natusch/D	-	
TU5	+	+
TU5/D	++	
WEHI-164	+	-
WEHI/D	++	-
Non-Transformed Targets		
Lymphocytes	-	
Lung	-	+
Lung/D	-	
RBC (human)	-	
RBC (Sheep)	-	

Figure Legends

Figure 1. Cytolytic activity of freshly isolated monocytes in 6-hr ^{51}Cr-release assays against WEHI/D. Merans of triplicate determinations are plotted at E:T = 5:1. Each line connects results from samples of a single monocyte preparation tested in plain assay media (negative for endotoxin by Limulus amebocyte lysate gelation) or in the presence of 10 µg/ml polymyxin B or 10 ng/ml LPS. Each box represents the mean ±1 standard deviation. The increase in cytolytic activity with LPS is highly significant ($p<0.001$, Wilcoxon two-tailed rank-sum test). (From Chen et al. ref. 52).

Figure 2. Effect of coculture with various cell lines on CM production by monocytes. Supernatants of 5×10^5 monocytes/ml incubated 5 hr with 5×10^5 cultured cells/ml in endotoxin-free media. Control supernatants were from monocytes incubated alone with or without 15 µg/ml of LPS. Cytolytic activity was measured in 6-hr assays against WEHI/D.

Figure 3. Effect of CM activity against WEHI/D of absorption by target cells. Aliquots of monocyte supernatant were incubated 1 hr at 4°C alone or with 1×10^6 cultured cells/ml.
Inset: Aliquots of monocyte supernatant were diluted 1:50 before incubation 1 hr at 4°C alone or with 2×10^7 cultured cells/ml.

Figure 4. Effect of polymyxin B on stimulation of CM production by LPS and by target cell conditioned media. Aliquots of 5×10^5 monocytes/ml

were incubated for 5 hr, with or without 10 µg/ml polymyxin B, in target cell conditioned media or fresh assay media containing 10 ng/ml LPS. Cytolytic activity in the resulting supernatants was measured in 6-hr assays against WEHI/D.

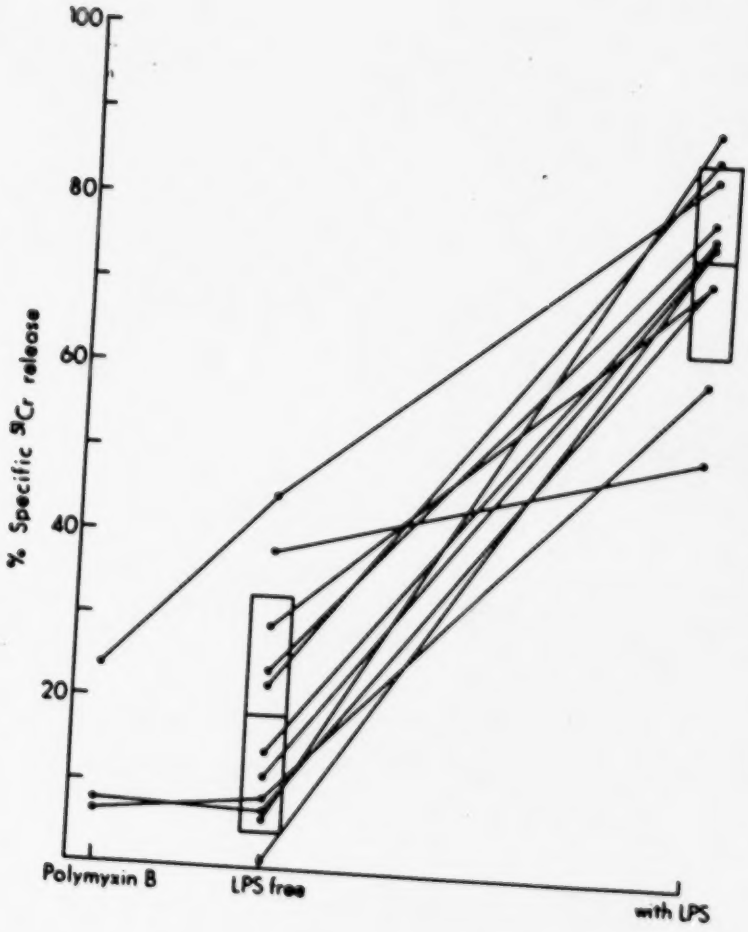
100
80
60
40
20
% Specific 51Cr release
Polymyxin B
LPS free
with LPS

Figure 1

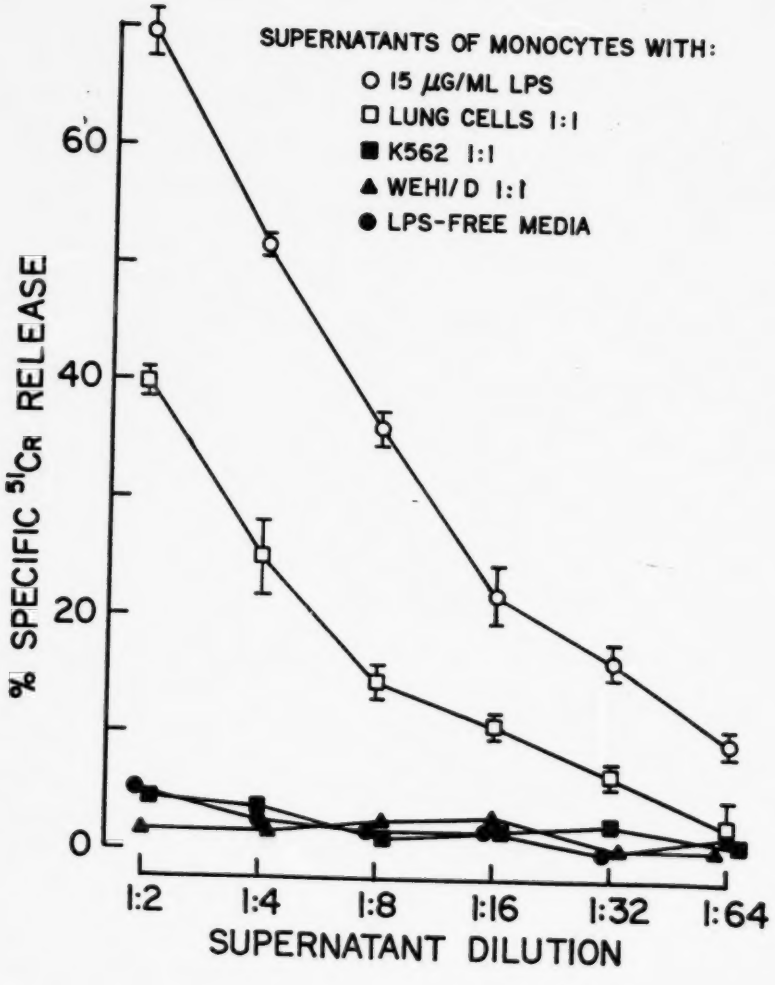
SUPERNATANTS OF MONOCYTES WITH:
O 15 μG/ML LPS
□ LUNG CELLS 1:1
■ K562 1:1
▲ WEHI/D 1:1
● LPS-FREE MEDIA
% SPECIFIC 51Cr RELEASE
60
40
20
0
1:2
1:4
1:8
1:16
1:32
1:64
SUPERNATANT DILUTION

Figure 2

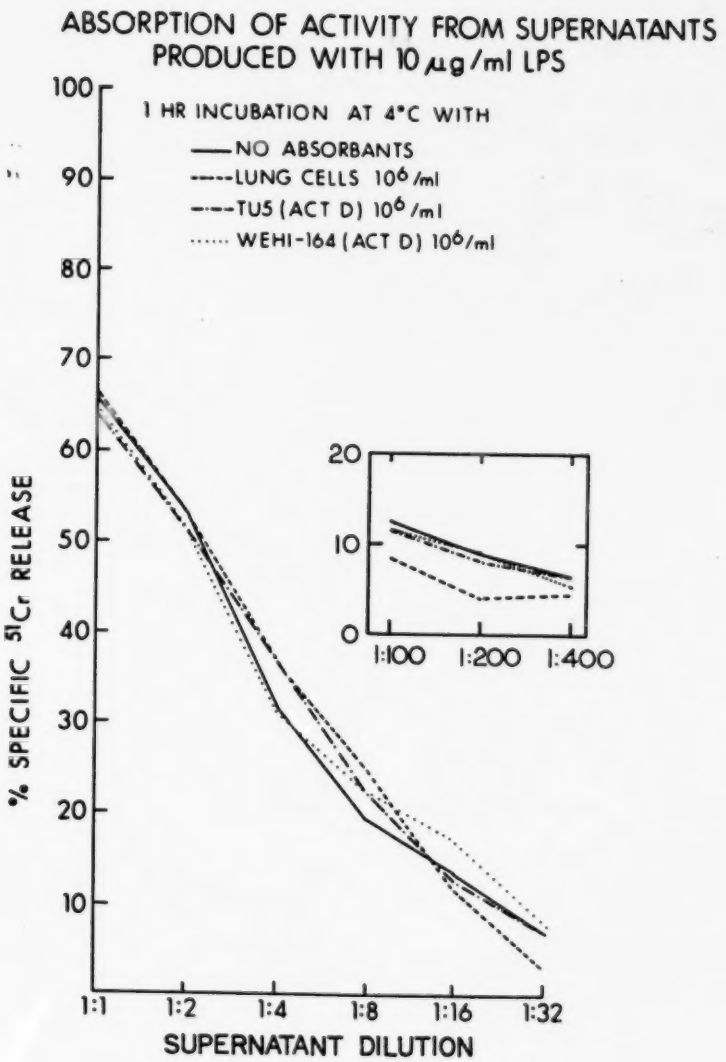
ABSORPTION OF ACTIVITY FROM SUPERNATANTS
PRODUCED WITH 10 μg/ml LPS
1 HR INCUBATION AT 4°C WITH
NO ABSORBANTS
LUNG CELLS 10⁶/ml
TU5 (ACT D) 10⁶/ml
WEHI-164 (ACT D) 10⁶/ml
% SPECIFIC ⁵¹Cr RELEASE
100
90
80
70
60
50
40
30
20
10
20
10
0
1:100
1:200
1:400
1:1
1:2
1:4
1:8
1:16
1:32
SUPERNATANT DILUTION

Figure 3

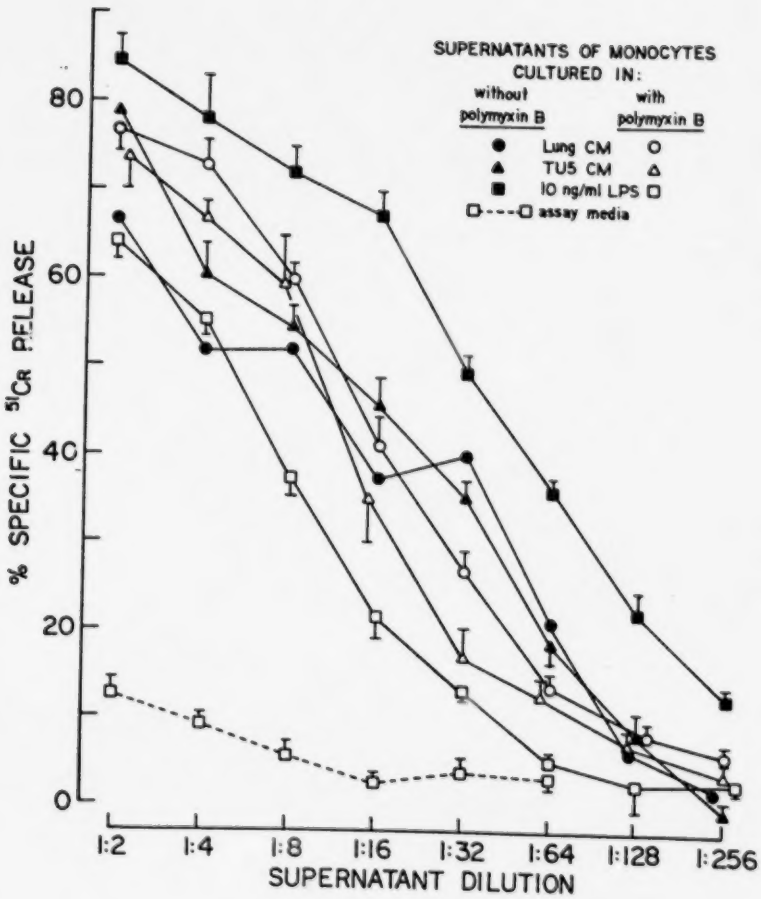
SUPERNATANTS OF MONOCYTES CULTURED IN:
without polymyxin B
with polymyxin B
Lung CM
TU5 CM
10 ng/ml LPS
assay media
% SPECIFIC 51Cr RELEASE
0
20
40
60
80
1:2
1:4
1:8
1:16
1:32
1:64
1:128
1:256
SUPERNATANT DILUTION

Figure 4